

COFFEE

1 ~~2~~ ~~1~~
2 2. The method of claim ~~1~~, wherein the determining comprises at least
3 partially separating the target analytes from other biological components in the sample
4 prior to determining the flux values.

1 ⁴~~A.~~ The method of claim ³~~2~~, wherein the plurality of capillary
2 electrophoresis methods are selected from the group consisting of capillary zone
3 electrophoresis, capillary isoelectric focusing and capillary gel electrophoresis.

1 ~~6~~⁶ The method of claim ~~6~~⁵, wherein the performing of the capillary
2 electrophoresis methods comprises performing a plurality of capillary zone
3 electrophoresis methods.

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73

4 polysaccharides, carbohydrates, nucleic acids, amino acids, nucleotides, nucleosides, fats,
5 fatty acids and organic acids.

1 ⁸8. The method of claim ³3, wherein the separating comprises
2 conducting a non-electrophoretic separation technique prior to conducting the plurality of
3 electrophoresis methods to precipitate at least some of the biological components.

1 ~~87~~ ⁹9. ~~The method of claim 1, wherein the stable isotope is selected from~~
2 ~~the group consisting of ¹³C, ²H and ¹⁵N, ¹⁸O, and ³⁴S.~~

1 ¹⁰10. The method of claim ¹1, wherein the substrate is selected from the
2 group consisting of proteins, carbohydrates, nucleic acids, amino acids, nucleotides,
3 nucleosides, fatty acids, organic acids, and fats.

1 ~~11~~ ¹⁰10. The method of claim ¹⁰10, wherein the substrate is a protein.

1 ~~12~~ ¹²12. The method of claim ¹1, wherein the substrate is a substrate for at
2 least two separate metabolic pathways in the subject, metabolism of the substrate via the
3 at least two metabolic pathways generating at least two byproducts, and wherein the target
4 metabolites comprise the at least two byproducts.

1 ~~13~~ ¹³13. The method of claim ¹1, wherein the sample is obtained from a
2 bodily fluid, the bodily fluid selected from the group consisting of blood, urine, cerebral
3 fluid, spinal fluid, sweat, and gastrointestinal fluids.

1 ~~14~~ ¹⁴14. The method of claim ¹1, wherein the sample is a cell, a tissue
2 sample or fecal material.

1 ~~15~~ ¹⁵15. The method of claim ¹1, wherein the determining comprises
2 obtaining multiple samples from the subject at different predetermined time points,
3 separating the target analytes from other biological components in each of the samples,
4 and determining the abundance of the isotope in the target analytes contained in each
5 sample, whereby a plurality of values for the abundance of the isotope in each target
6 analyte are obtained, the flux value for each target analyte being determined from the
7 plurality of abundance values determined for it.

1 ~~16~~¹⁶ 16. The method of claim ~~1~~¹, wherein the target analytes are selected
 2 from the group of proteins, carbohydrates, nucleic acids, amino acids, nucleotides,
 3 nucleosides, fatty acids, organic acids, and fats.

1 ~~17~~¹⁷ 17. The method of claim ~~16~~¹⁶, wherein the target analyte is a protein.

1 ~~18~~¹⁸ 18. The method of claim ~~1~~¹, wherein the plurality of target analytes
 2 comprise the substrate and at least one target metabolite.

1 ~~19~~¹⁹ 19. The method of claim ~~1~~¹, wherein the plurality of target analytes is at
 2 least 3 target metabolites.

1 ~~20~~²⁰ 20. The method of claim ~~19~~¹⁹, wherein the plurality of target analytes is
 2 at least 5 target metabolites.

1 ~~21~~²¹ 21. The method of claim ~~1~~¹, wherein determination of the abundance of
 2 the isotope is performed by mass spectrometry, infrared spectrometry or nuclear magnetic
 3 resonance spectrometry.

1 ~~22~~²² 22. The method of claim ~~21~~²¹, wherein determination of the abundance
 2 of the isotope is performed by mass spectrometry.

1 ~~A3 > 23~~²³ ~~23. The method of claim ~~2~~², wherein~~
 2 (a) the stable isotope is ^{13}C ;
 3 (b) separating comprises performing a plurality of capillary
 4 electrophoresis methods, wherein the plurality of electrophoresis methods are selected
 5 from the group consisting of capillary zone electrophoresis, capillary isoelectric focusing
 6 ~~and capillary gel electrophoresis; and~~
 7 (c) the determination of the abundance of the isotope is
 8 performed by mass spectrometry.

1 ~~24~~²⁴ 24. A method for analyzing metabolic pathways, comprising:
 2 (a) separating at least partially a plurality of target analytes
 3 from biological components contained in a sample obtained from a subject, the target
 4 analytes comprising a substrate labeled with a stable isotope and/or one or more target

5 metabolites resulting from the metabolism of the substrate by the subject, and wherein the
6 relative isotopic abundance of the isotope in the substrate is known; and

7 (b) determining the abundance of the isotope in a plurality of
8 the target analytes in the sample to determine a value for the flux of each target analyte.

1 ²⁵
~~25.~~ The method of claim ²⁵24, wherein the separating comprises
2 performing a plurality of capillary electrophoresis methods in series, the capillary
3 electrophoresis methods selected from the group consisting of capillary zone
4 electrophoresis, capillary isoelectric focusing and capillary gel electrophoresis.

1 ²⁶
~~26.~~ The method of claim ²⁵25, wherein determination of the abundance
2 of the isotope is performed by mass spectrometry.

1 ²⁷
~~27.~~ A method for screening for metabolites correlated with a disease,
2 comprising:

3 (a) administering to a test subject and a control subject a substrate
4 labeled with a stable isotope, wherein the relative isotopic abundance of the isotope in the
5 substrate is known and the test subject has the disease;

6 (b) allowing the labeled substrate to be at least partially metabolized
7 by the test subject and control subject to form one or more target metabolites, and wherein
8 the conditions under which the administering and allowing steps are performed ~~are~~ the
9 same for the test and control subject; and

10 (c) obtaining a sample from the test and control subject;

11 (d) determining for each sample the relative abundance of the isotope
12 in a plurality of target analytes to determine a value for the flux of each target analyte,
13 wherein the target analytes comprise the substrate and/or one or more of the target
14 metabolites; and

15 (e) comparing the values for flux for the test and control subjects, a
16 difference in the flux value for a target analyte in the test subject and corresponding flux
17 value for the control subject indicating that such analyte is potentially correlated with the
18 disease.

1 ²⁸
~~28.~~ The method of claim ²⁷27, wherein the determining step comprises at
2 least partially separating the target analytes from other biological components in the

3 sample prior to determining the flux values, the separating comprising separately
4 performing a plurality of capillary electrophoresis methods in series with the samples
5 from the test and control subjects.

1 ~~29~~²⁸ The method of claim ~~29~~²⁸, wherein the determination of the isotopic
2 abundance is performed by mass spectrometry.

1 ~~30~~²⁷ The method of claim ~~30~~²⁷, wherein the disease is selected from the
2 group consisting of cancer, autism, microbial infection and digestive disorders.

1 ~~31~~³¹ A method for screening for metabolites correlated with a disease,
2 comprising:

3 (a) analyzing a sample from a test subject having the disease, the
4 sample comprising a substrate labeled with a stable isotope administered to the test
5 subject and/or one or more target metabolites resulting from metabolism of the substrate
6 by the test subject, the relative isotopic abundance of the isotope in the substrate known at
7 the time of administration, and wherein the analyzing step comprises determining the
8 isotopic abundance of the isotope in a plurality of analytes in the sample to determine a
9 value for the flux of each analyte, wherein the plurality of analytes comprise the substrate
10 and/or one or more of the target metabolites; and

11 (b) comparing flux values for the analytes with flux values for the
12 same analytes obtained for a control subject, wherein a difference in a flux value for an
13 analyte indicates that such analyte is correlated with the disease.

- 1 ~~32~~³² A method for screening for the presence of a disease, comprising:
- 2 (a) administering to a test subject a substrate labeled with a stable
- 3 isotope, wherein the relative abundance of the isotope in the substrate is known;
- 4 (b) allowing sufficient time for the labeled substrate to be at least
- 5 partially metabolized by the test subject to form one or more target metabolites known to
- 6 be correlated with the disease;
- 7 (c) performing a plurality of electrophoretic methods in series to at
- 8 least partially separate a plurality of target analytes from other biological components in a
- 9 sample obtained from the test subject, wherein the target analytes comprise the substrate
- 10 and/or one or more of the target metabolites;
- 11 (d) determining a flux value for the target analytes, the flux value for
- 12 each target analyte being determined from the abundance of the isotope in that analyte;
- 13 and
- 14 (e) comparing determined flux values with corresponding reference
- 15 flux values for the same target analytes to assess the test subject's risk of disease.

- 1 ~~33~~³³ The method of claim ~~32~~³², wherein
- 2 (i) if the reference flux values are representative of presence and/or
- 3 susceptibility to the disease, a statistically significant difference between reference values
- 4 and test values indicates that the test subject does not have and/or is not susceptible to
- 5 acquiring the disease; and
- 6 (ii) if the reference flux values are representative of absence and/or lack of
- 7 susceptibility to the disease, a statistically significant difference between reference values
- 8 and test values indicates that the test subject does have, or is susceptible to acquiring, the
- 9 disease.

- 1 ~~34~~³⁴ The method of claim ~~33~~³³, wherein the plurality of electrophoretic
- 2 methods are selected from the group consisting of capillary gel electrophoresis, capillary
- 3 zone electrophoresis and capillary gel electrophoresis.

- 1 ~~35~~³⁵ A method for screening for the presence of a disease, comprising:
- 2 (a) analyzing a sample from a test subject, the sample comprising a
- 3 substrate labeled with a stable isotope administered to the test subject and/or one or more

4 target metabolites resulting from metabolism of the substrate by the test subject, the
5 relative isotopic abundance of the isotope in the substrate known at the time of
6 administration, and wherein the analyzing step comprises determining the abundance of
7 the isotope in a plurality of analytes in the sample to determine a value for the flux of
8 each analyte, wherein the plurality of analytes comprise the substrate and/or one or more
9 of the target metabolites; and

10 (b) for each target analyte, comparing the determined flux value with a
11 range of flux values for that analyte, wherein the range is known to be correlated with the
12 disease and ~~a~~ a determined flux value for a target analyte ^{falling} ~~is~~ within the range for that
13 target analyte, ~~it~~ indicates that the test subject has the disease or is susceptible to the
14 disease.

1 ³⁶
~~36.~~ A method for analyzing metabolites in an initial sample,
2 comprising
3 (a) performing a plurality of capillary electrophoresis methods in
4 series, each method comprising electrophoresing a sample containing multiple
5 metabolites, whereby a plurality of resolved metabolites are obtained, and wherein
6 (i) the sample electrophoresed contains only a subset of the
7 plurality of resolved metabolites from the immediately preceding method in the series,
8 except the first method of the series in which the sample is the initial sample, the
9 metabolites in the initial sample potentially containing one or more target analytes;
10 (ii) the capillary electrophoresis methods are selected from the
11 group consisting of capillary isoelectric focusing electrophoresis, capillary zone
12 electrophoresis and capillary gel electrophoresis; and
13 (b) analyzing fractions containing resolved metabolites from the final
14 electrophoretic method to detect the presence of the target analytes.

1 ³⁷
~~37.~~ The method of claim ³⁶~~36~~, wherein the one or more target analytes
2 are labeled with an isotopic label, and the analyzing comprises detecting the abundance of
3 the label in each target analyte present.

1 ³⁸
~~38.~~ The method of claim ³⁷~~37~~, wherein the analyzing is performed by
2 mass spectroscopy, infrared spectroscopy or nuclear magnetic resonance spectroscopy.